

CLAIMS

WE CLAIM:

- 5 1. A method for the treatment of a skin defect comprising the application, to said defect, of a portion of an epidermal or complex skin equivalent comprising keratinocyte precursor cells derived from the culturing of outer root sheath cells which were initially derived from the culturing, *in toto*, of an anagen or growing hair.
- 10 2. The method of claim 1, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.
- 15 3. The method of claim 1, wherein said outer root sheath cells are homologous cells.
4. The method of claim 1, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
- 20 5. The method of claim 1, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing human serum in a concentration of less than 5%.
- 25 6. The method of claim 1, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
7. The method of claim 1, wherein said epidermal or skin equivalents are coated on their top or cornified side with a fibrin glue.
- 30 8. The method of claims 1 or 7, wherein said epidermal or skin equivalents are coated on their top or cornified side with a carrier membrane.

- 5 9. A method for the treatment of a skin defect comprising the application, to said defect, of a portion of an epidermal or skin equivalent comprising keratinocyte precursor cells derived from the culturing of outer root sheath cells which were initially derived from the culturing of an anagen or growing hair; wherein all culturing of cells is performed in a medium which utilizes autologous or homologous human serum in a concentration of less than about 5%.
- 10 10. The method of claim 9, wherein said anagen or growing hair is cultured *in toto*.
11. The method of claim 9, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.
- 15 12. The method of claim 9, wherein said outer root sheath cells are homologous cells.
13. The method of claim 9, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
- 20 14. The method of claim 9, wherein said epidermal or skin equivalents are coated on their top or cornified side with a fibrin glue.
15. The method of claims 9 or 14, wherein said epidermal or skin equivalents are coated on their top or cornified side with a carrier membrane.

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16. A method for the treatment of a skin defect comprising the application, to said defect, of a portion of an epidermal or skin equivalent comprising keratinocyte precursor cells derived from the culturing of outer root sheath cells which were initially derived from the culturing of an anagen or growing hair; wherein said epidermal or skin equivalent are coated on their top or cornified side with a fibrin glue.
17. The method of claim 16, wherein said anagen or growing hair is cultured *in toto*.
18. The method of claim 16, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.
19. The method of claim 16, wherein said outer root sheath cells are homologous cells.
20. The method of claim 16, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
21. The method of claim 16, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing human serum in a concentration of less than 5%.
22. The method of claim 16, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
23. The method of claim 16, wherein said epidermal or skin equivalents are coated on their top or cornified side with a fibrin glue which contains one or more anti-microbial, anti-fungal, or anti-viral agents emulsified therein.

24. A method for the selection of keratinocyte precursor cells from the outer root sheath of hair for subsequent use in a composition for healing a skin defect, comprising the steps of:
- 5 (a) plucking of an anagen or growing hair;
- (b) primary-culturing the outer root sheath-derived keratinocyte precursor cells by adhering said anagen hair, *in toto*, to a microporous membrane, which possesses growth-arrested/limited feeder cells on its undersurface so as to select for keratinocyte precursor cells from the outer root sheath of
- 10 hair;
- (c) organotypically-culturing the outer root sheath cells harvested from said primary cultures by inoculating a microporous membrane which also possesses growth-arrested/limited feeder cells on its undersurface;
- (d) generating an epidermal or complex skin equivalent, for subsequent use as a graft insert, comprised of keratinocyte precursor cells by placing a
- 15 carrier membrane on top of said organotypic-culture from step (c) and detaching said skin or epidermal equivalent, which is comprised of the keratinocyte precursor cells and carrier membrane, together as a single, laminar unit;
- 20 (e) contacting said epidermal or skin equivalent with a skin defect present on an individual, and immobilizing said epidermal or skin equivalent at the site of contact.
25. The method of claim 24, wherein said outer root sheath cells are autologous cells derived from the individual who will subsequently undergo treatment for a skin defect.
26. The method of claim 24, wherein said outer root sheath cells are homologous cells.

27. The method of claim 24, wherein the culture density of said keratinocyte precursor cells is between about 3×10^4 cells/cm² and about 1×10^5 cells/cm².
28. The method of claim 24, wherein the culture density of said growth-arrested/limited feeder cells on said microporous membrane is between about 1×10^4 cells/cm² and about 5×10^4 cells/cm².
29. The method of claim 24, wherein said growth-arrested/limited feeder cells are banked or immortalized cells.
30. The method of claim 24, wherein said primary and organotypic cultures utilize autologous or homologous human serum.
31. The method of claims 24 or 30, wherein said primary and organotypic cultures utilize autologous or homologous human serum in a concentration of less than about 5%.
32. The method of claim 24, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous biological supplements.
33. The method of claim 24, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components.
34. The method of claims 24 or 33, wherein said epidermal or skin equivalent comprises outer root sheath cells cultured in a medium containing only homologous or autologous releasates from blood components at a concentration of about 0.1% to about 20%.

35. The method of claim 24, wherein said epidermal equivalents are coated on their top or cornified side with a fibrin glue.
36. The method of claims 24 or 35, wherein said epidermal equivalents are coated on their top or cornified side with a carrier membrane.
37. The method of claim 24, wherein said microporous membrane is coated by one or more extracellular matrix substances selected from a group consisting of: fibrin, fibronectin, collagens, laminins and hyaluronan.
38. The method of claims 24 or 37, wherein said microporous membrane possesses a growth-arrested/limited feeder cell system on its undersurface with said feeder cells of at least one type of cell selected from the group comprising human dermal fibroblasts, epidermal cells, mesenchymal cells, neuronal cells and endothelial cells.
39. The method of claim 24, wherein said carrier membrane is made from one or more types of materials selected from the group comprising polyester, PTFE, polyurethane, hyaluronic acid, polylactic acid, collagen, or a silicone or vaseline gauze dressing.
40. The method of claim 24, wherein the size of said epidermal equivalent is selected from the group consisting of 1.0 cm, 1.5 cm, 2.0 cm, and 2.5 cm in diameter.
41. A method of shipping or transporting epidermal equivalents comprising:
(a) detaching said epidermal equivalents from a culture medium, and
(b) transferring said epidermal equivalents onto a transport medium.
42. The method of claim 41, wherein said epidermal equivalents are coated on their top or cornified side with a carrier membrane.

43. The method of claim 42, wherein said epidermal equivalents are further sealed and shipped for future use in grafting.
- 5 44. The method of claim 41, wherein said transport medium comprises a solidified or gelled medium.
45. The method of claim 44, wherein said solidified or gelled medium is selected from the group consisting of agarose, methyl cellulose, or another gelifying substance.
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